

1 / 30

FIG. 1a

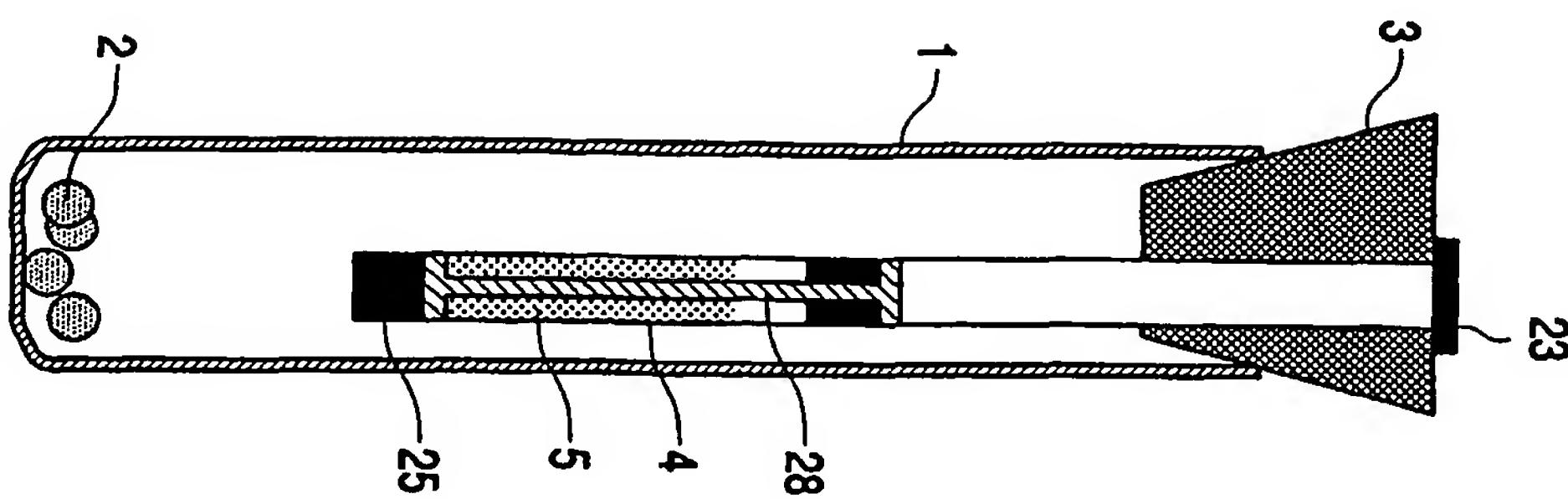


FIG. 1b

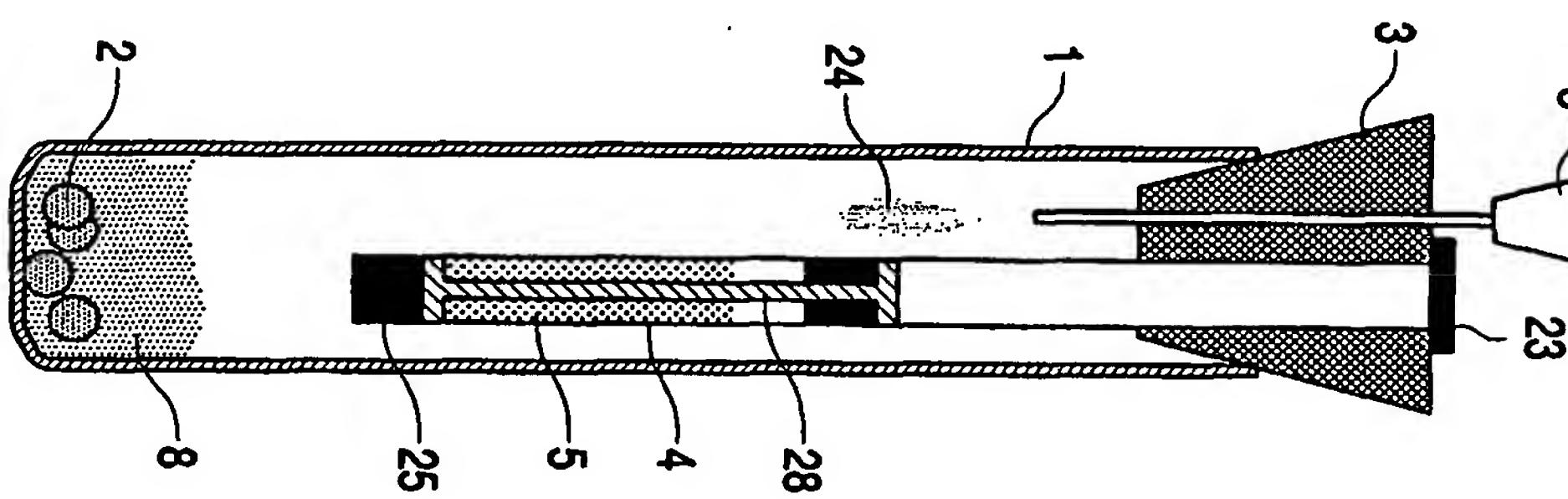


FIG. 1c

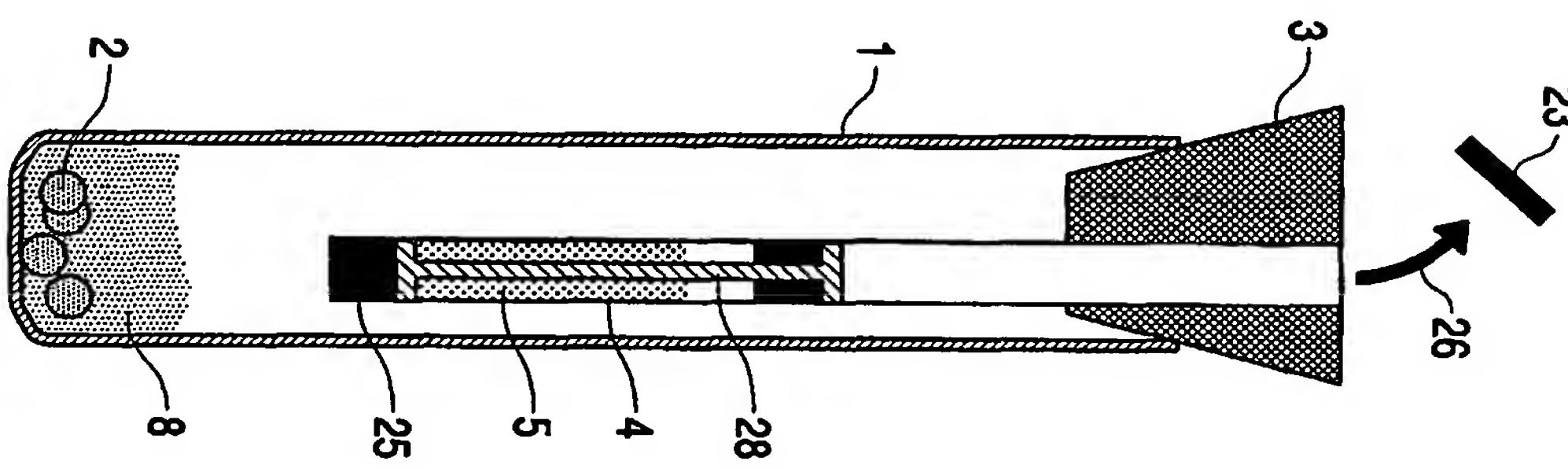
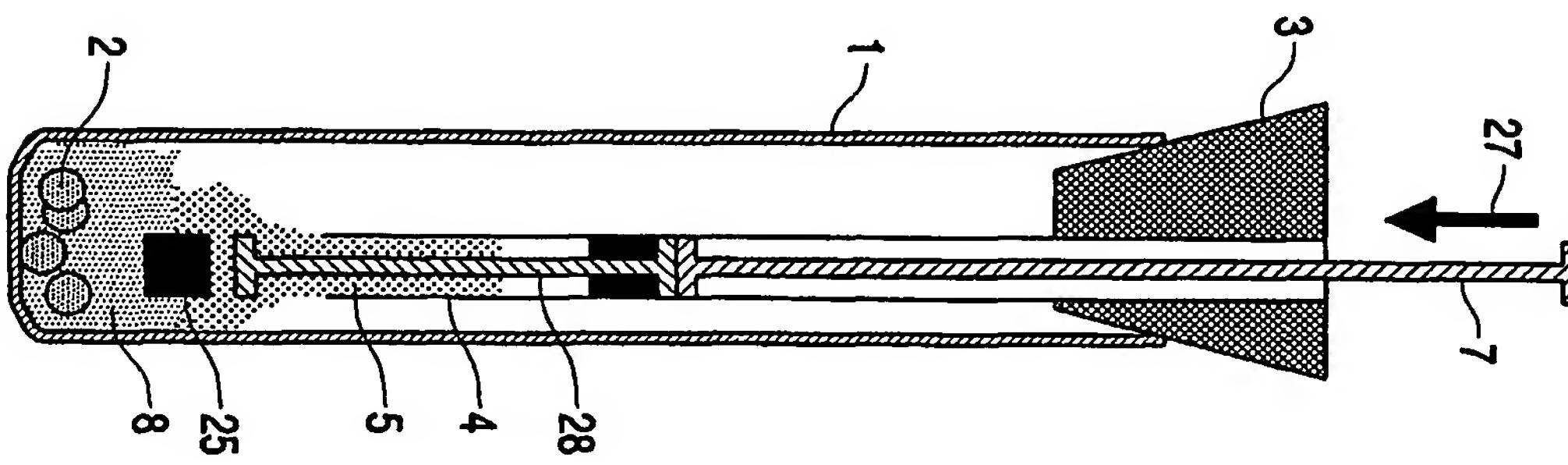
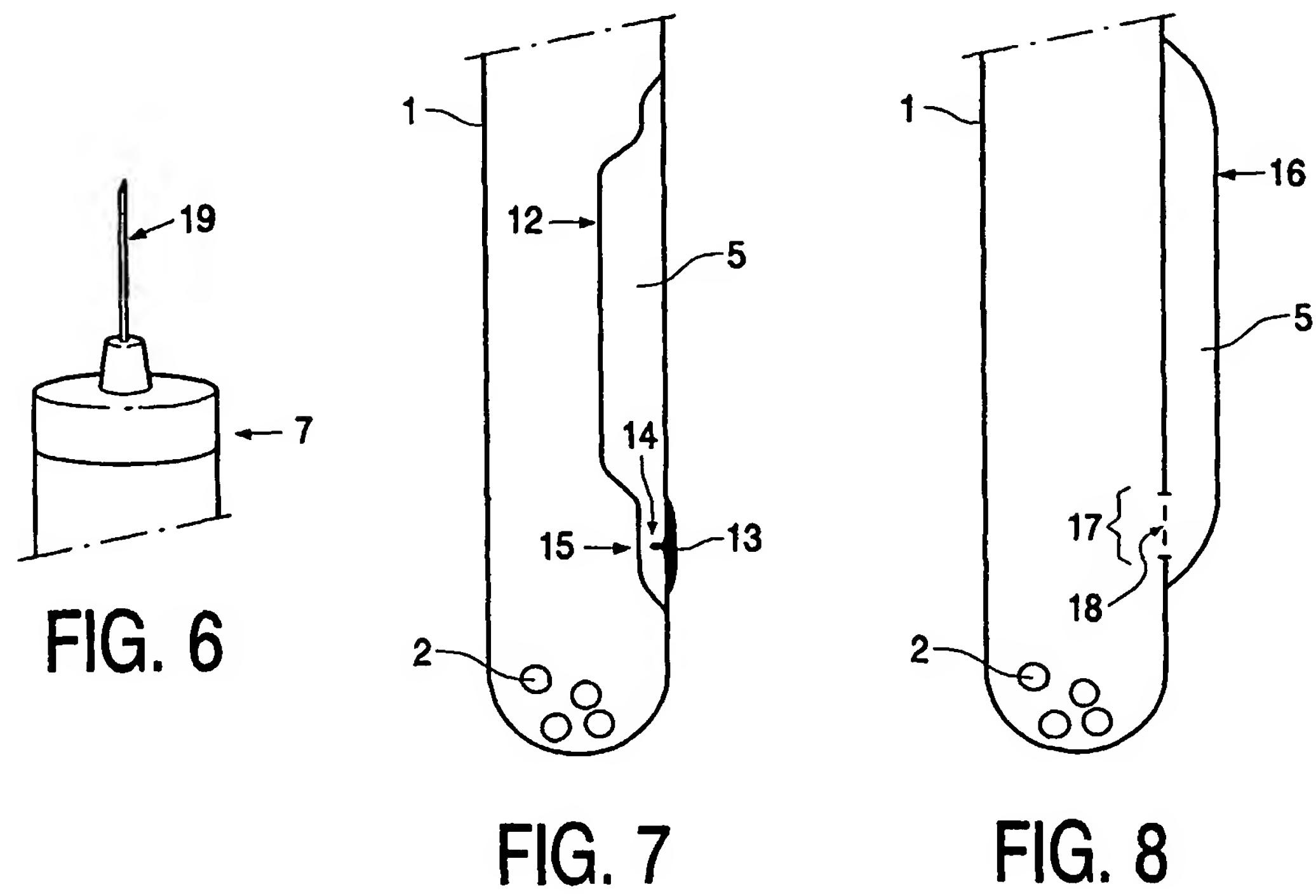
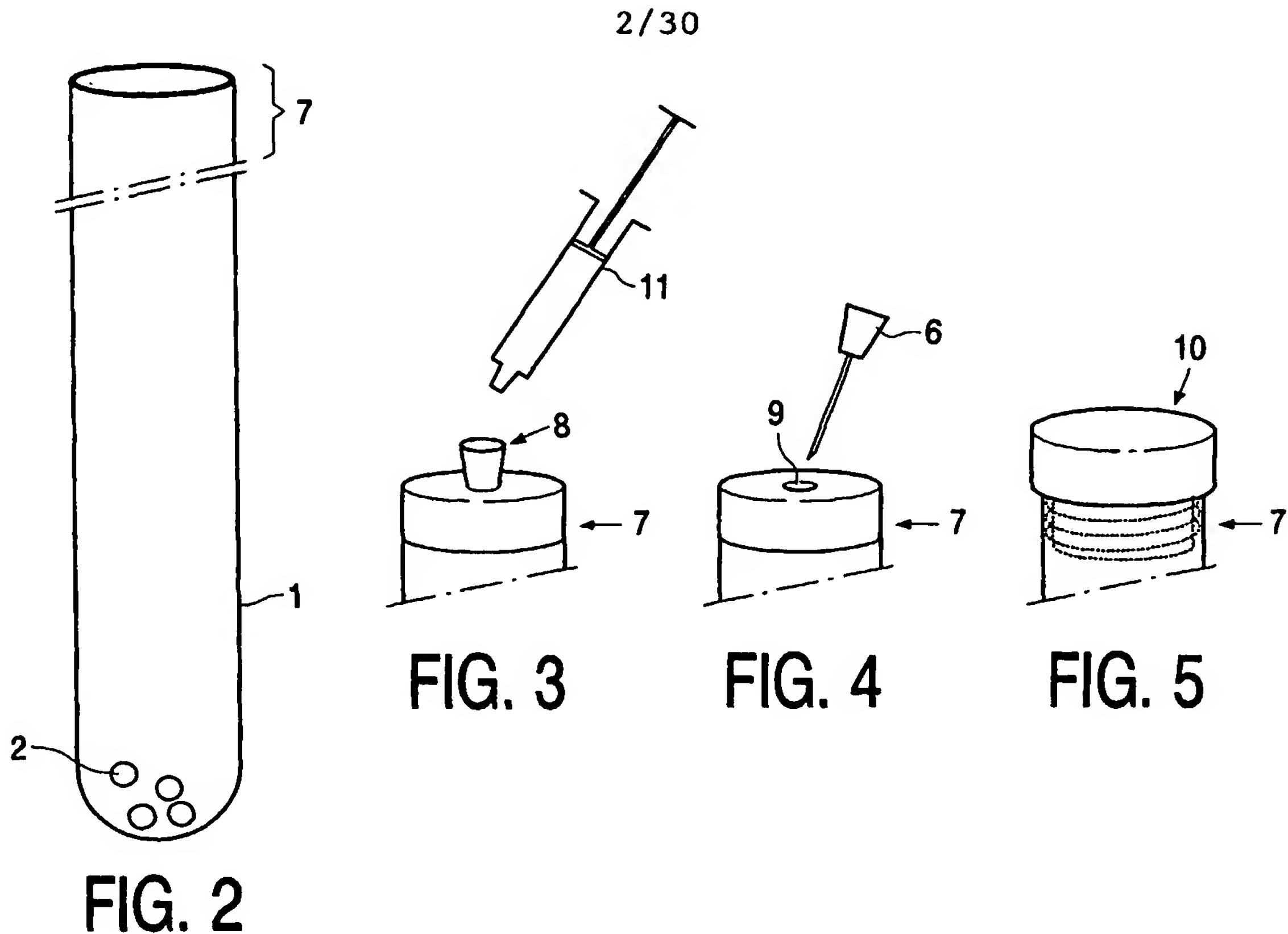


FIG. 1d





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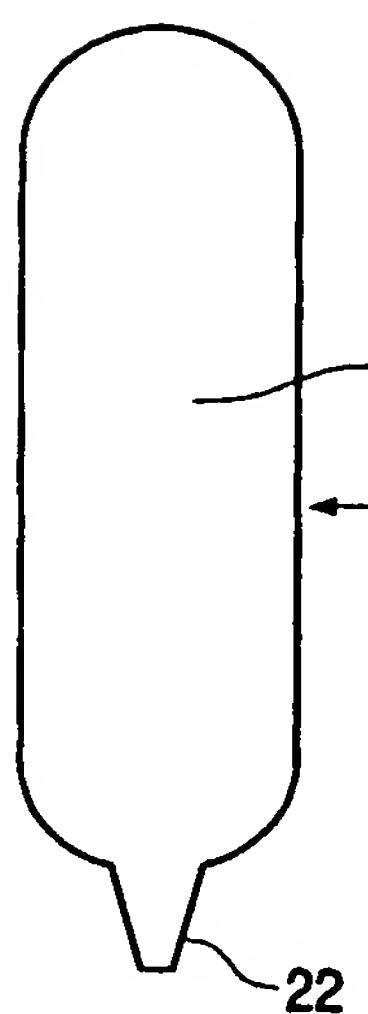


FIG. 9

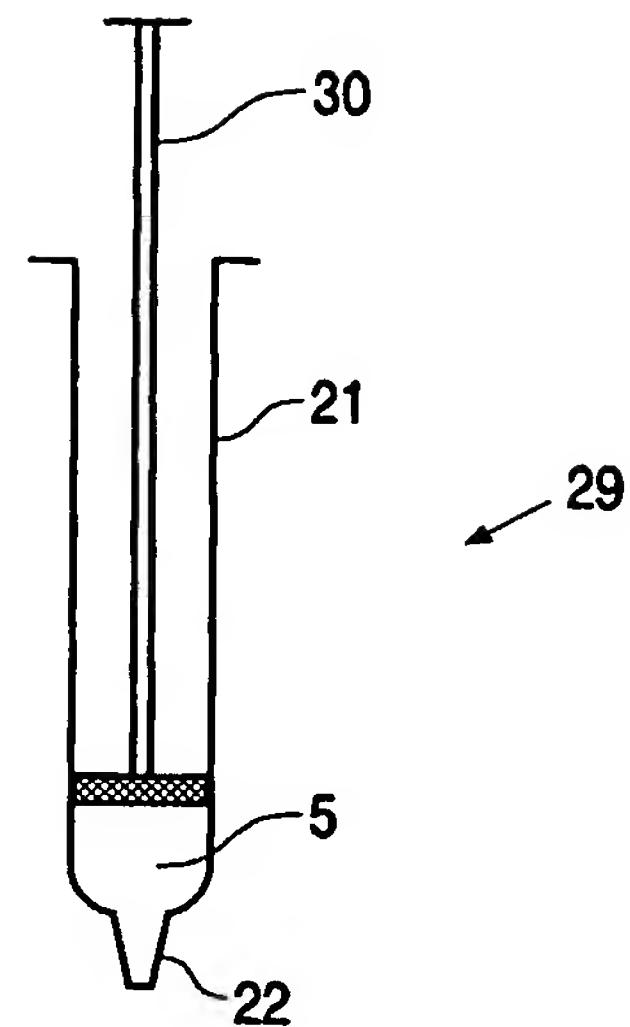


FIG. 10

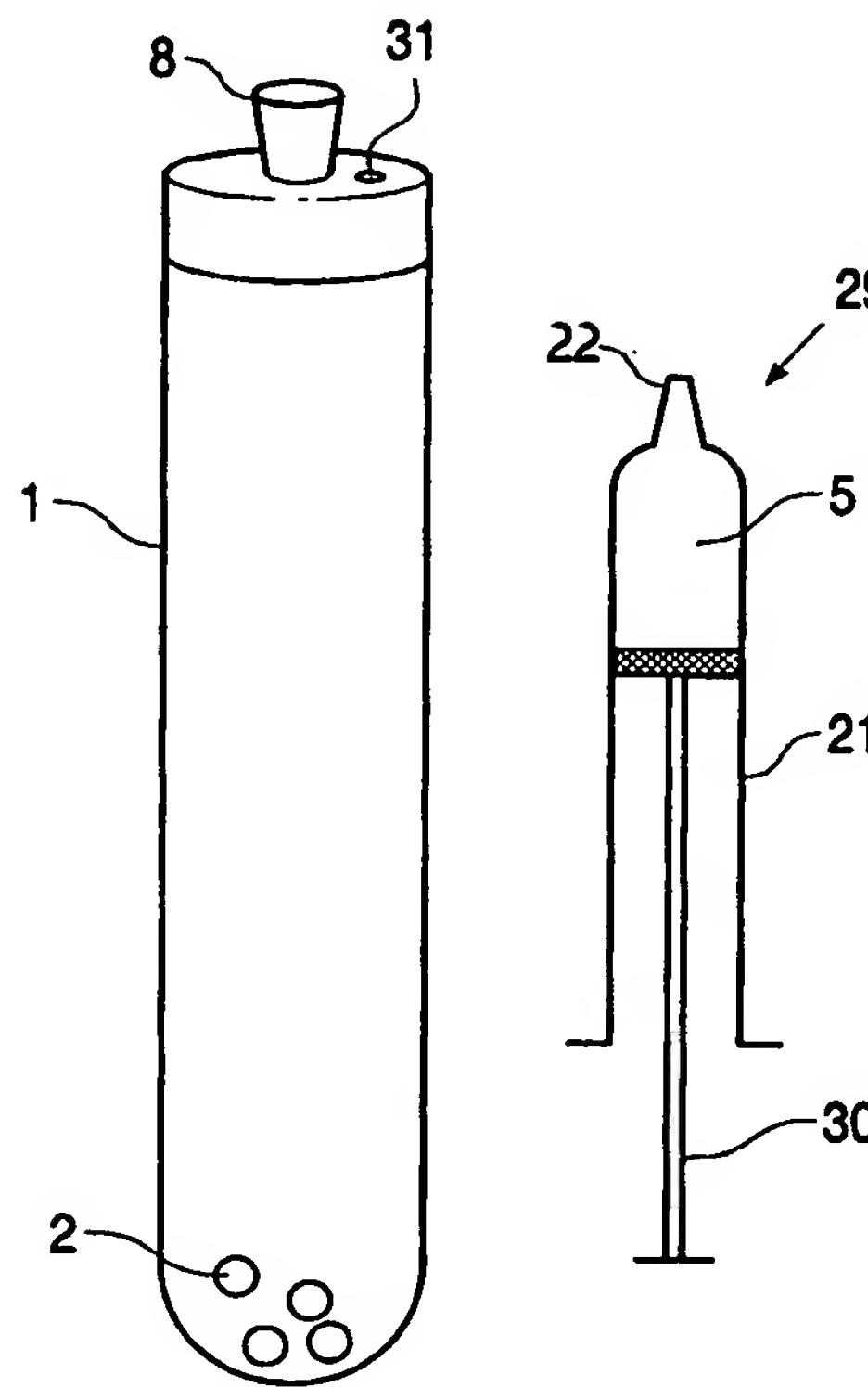


FIG. 11

Figure 12. Strategies followed in the given examples

Figure 12.1 *Ex vivo* monitoring of immune response against tetanus toxoid.

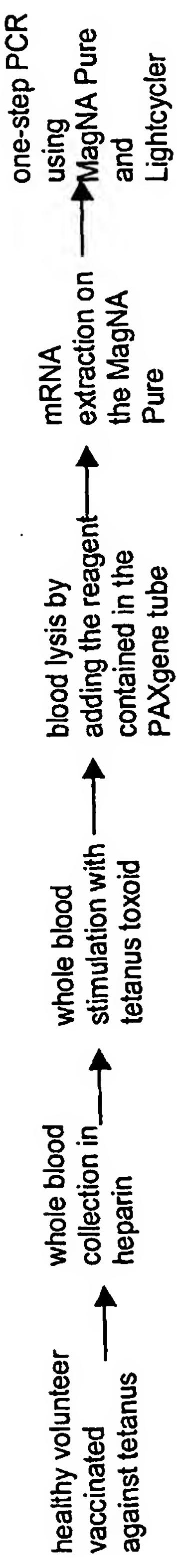
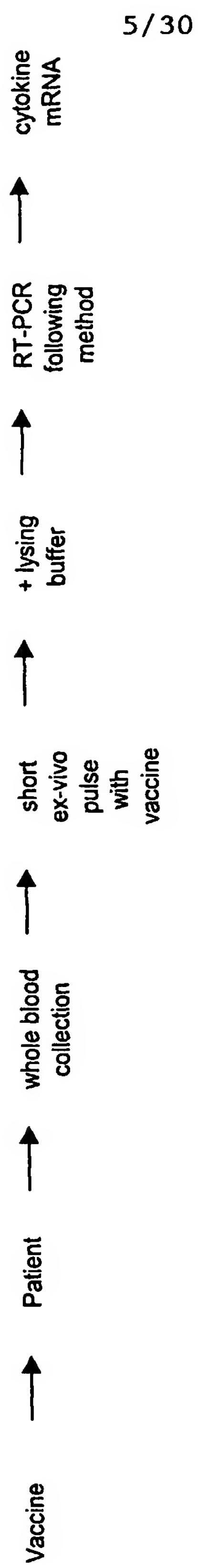


Figure 12.2 Strategy followed in example 3.



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Figure 12.3 Strategy followed in example 4

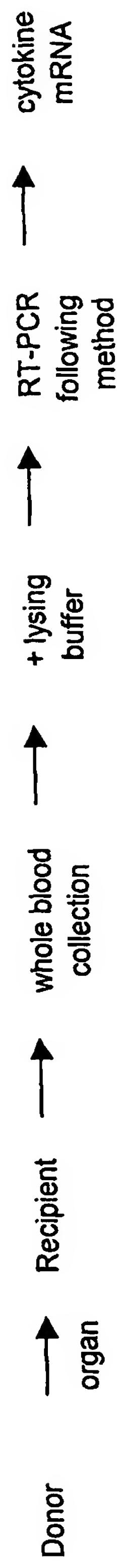
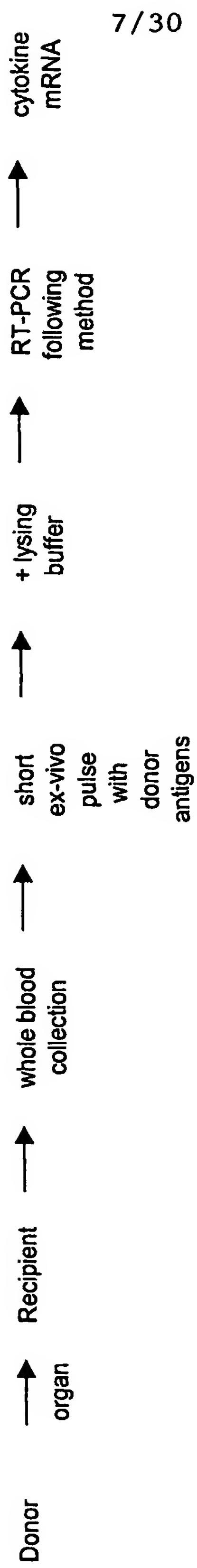


Figure 12.4 Strategy followed in example 5.



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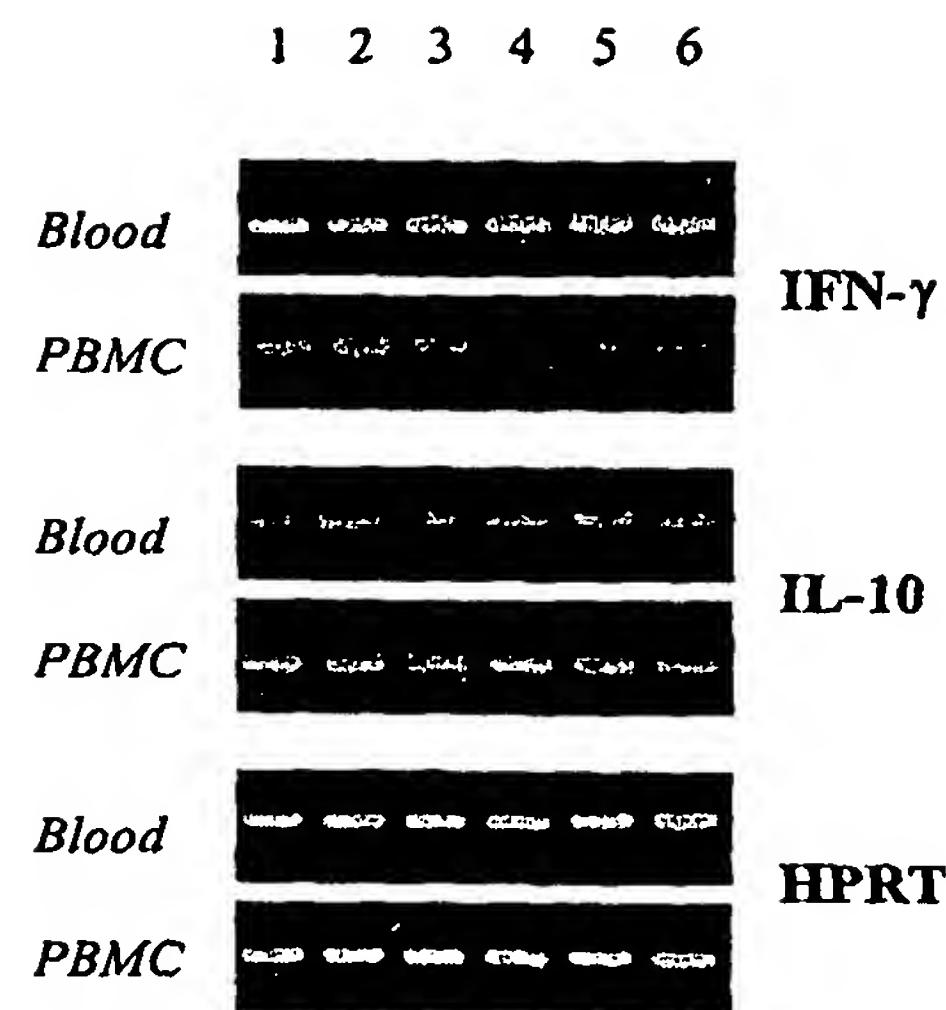


Figure 13.1

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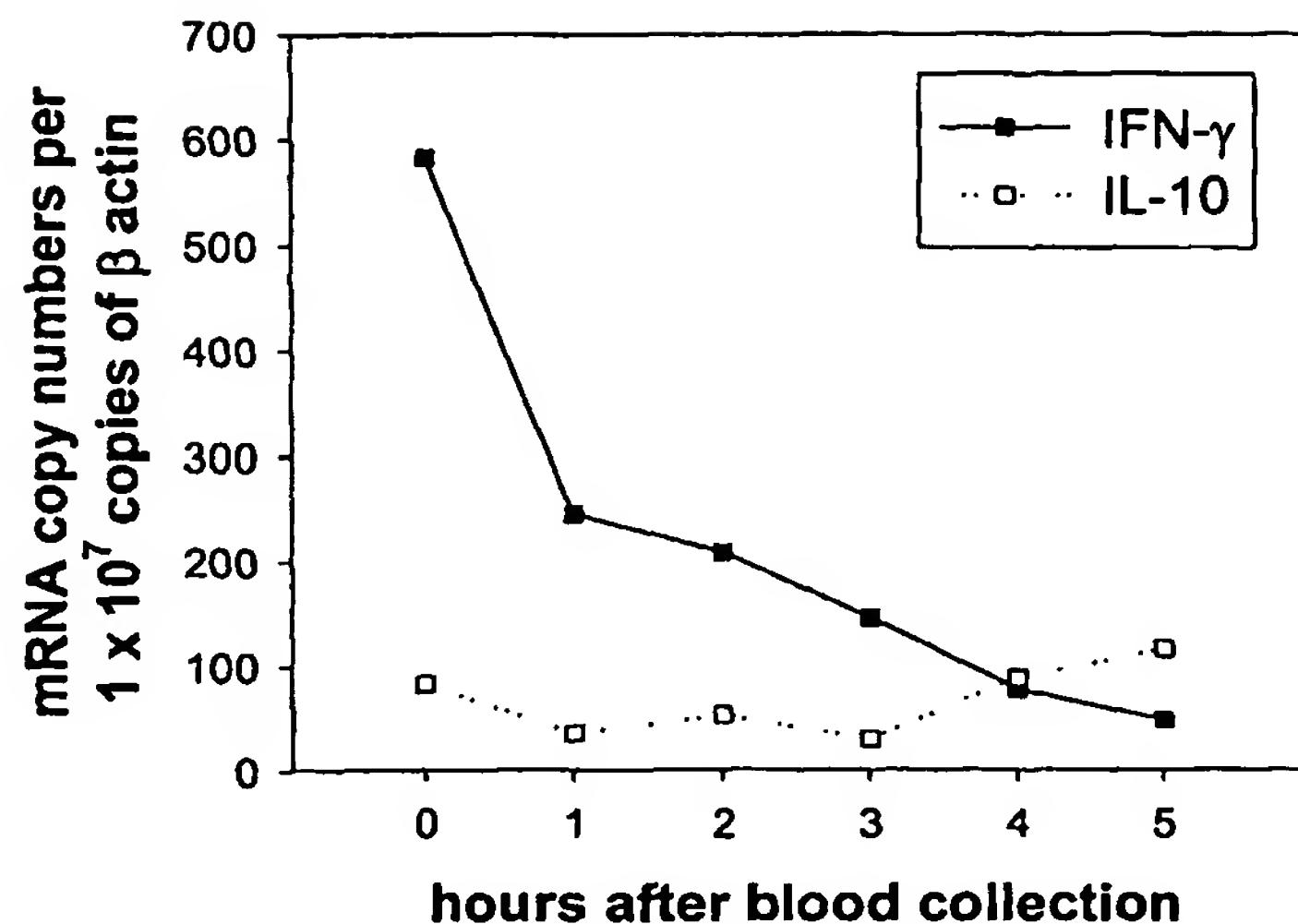


Figure 13.2

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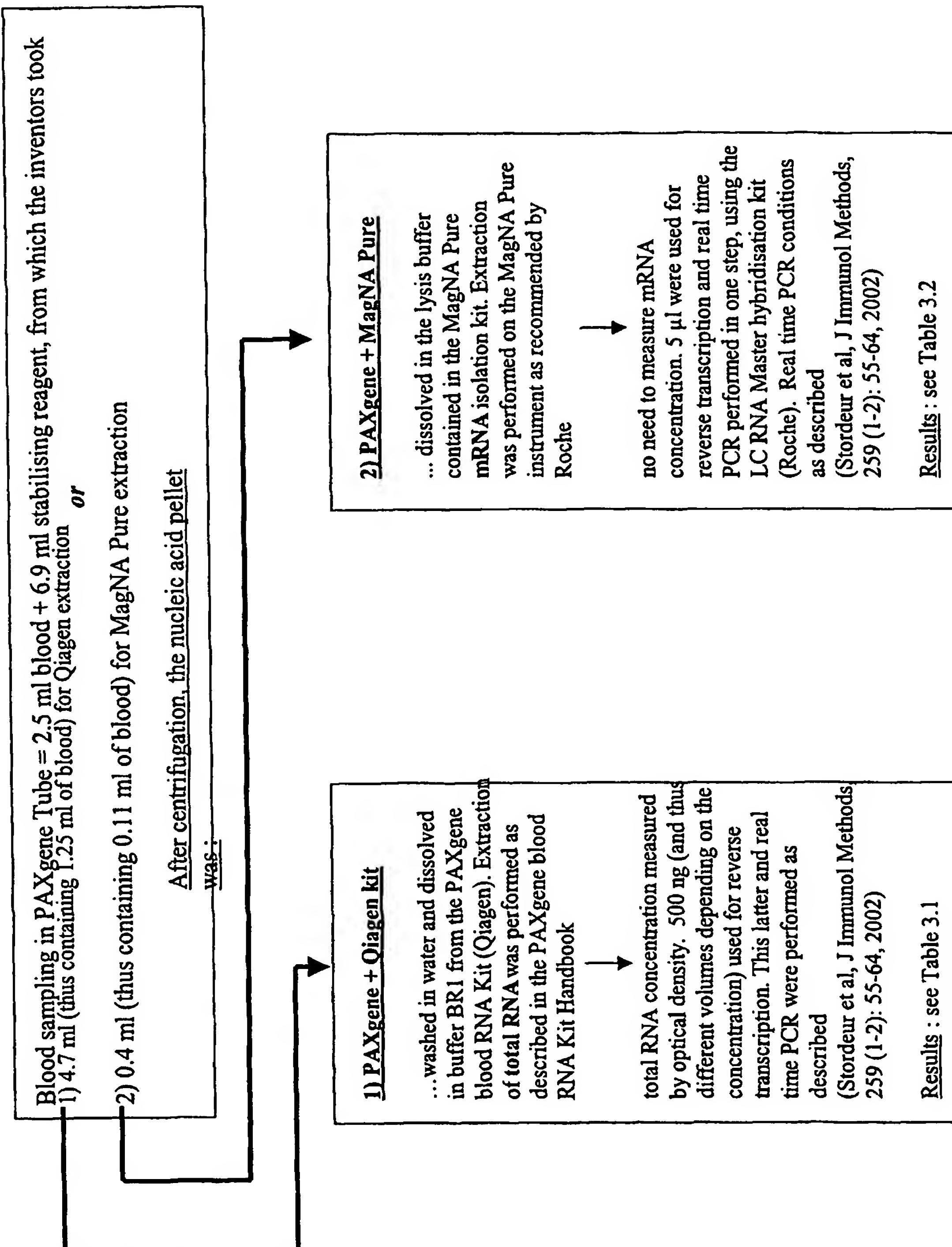


Figure 14

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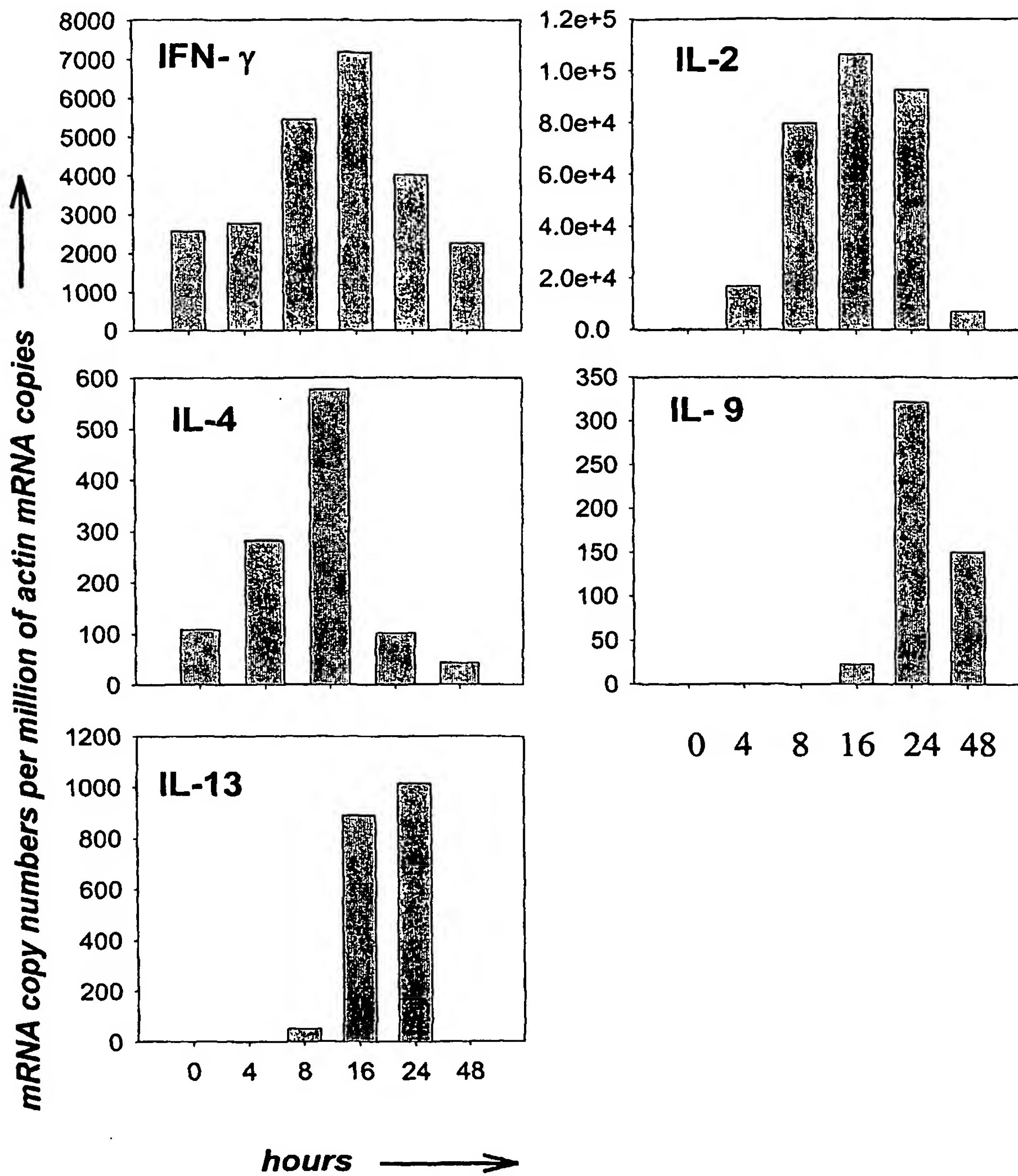


Figure 15

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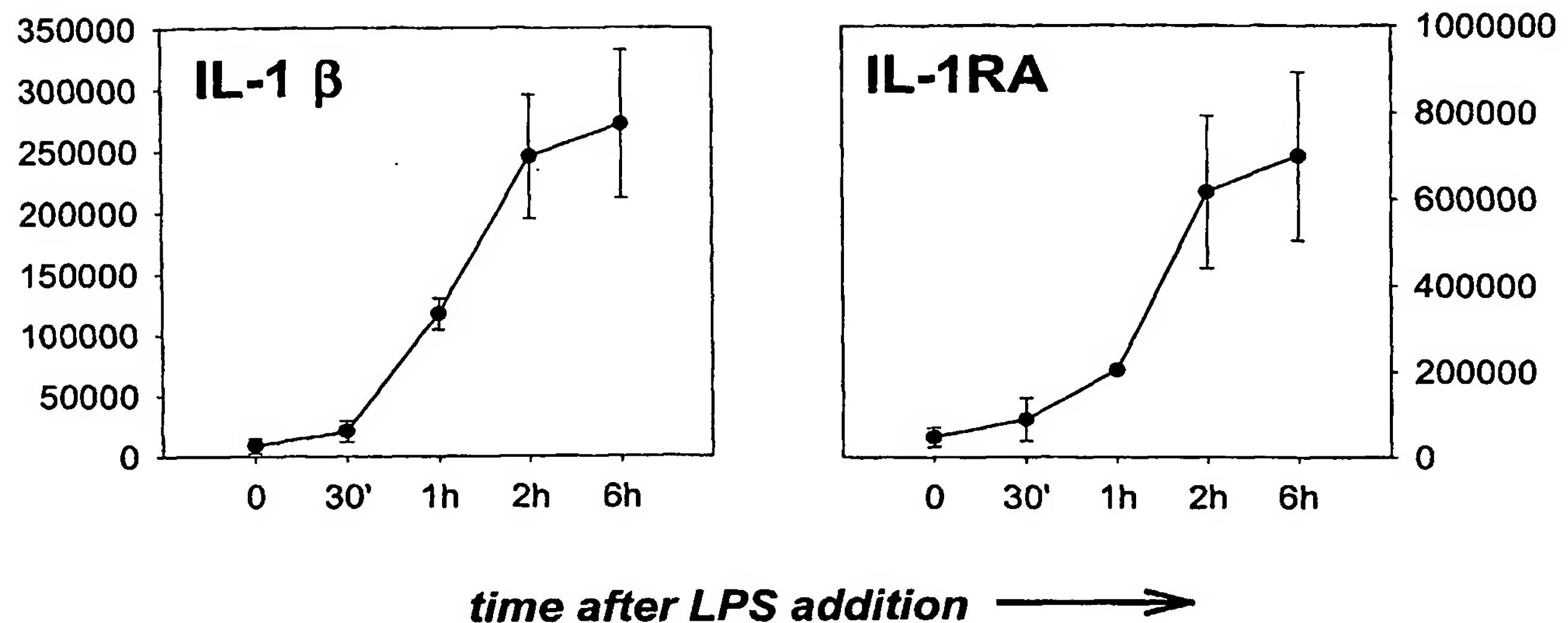


Figure 16

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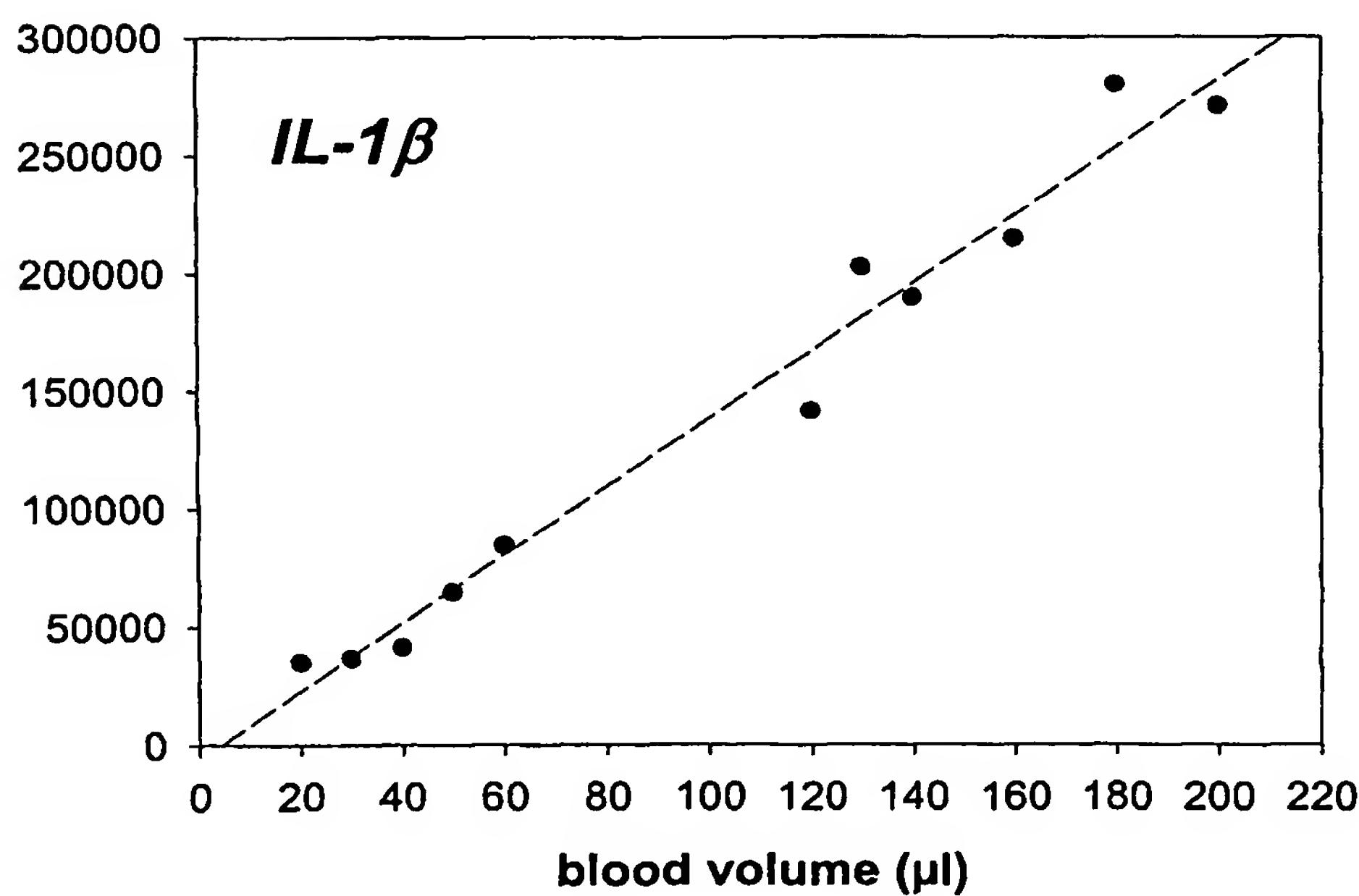
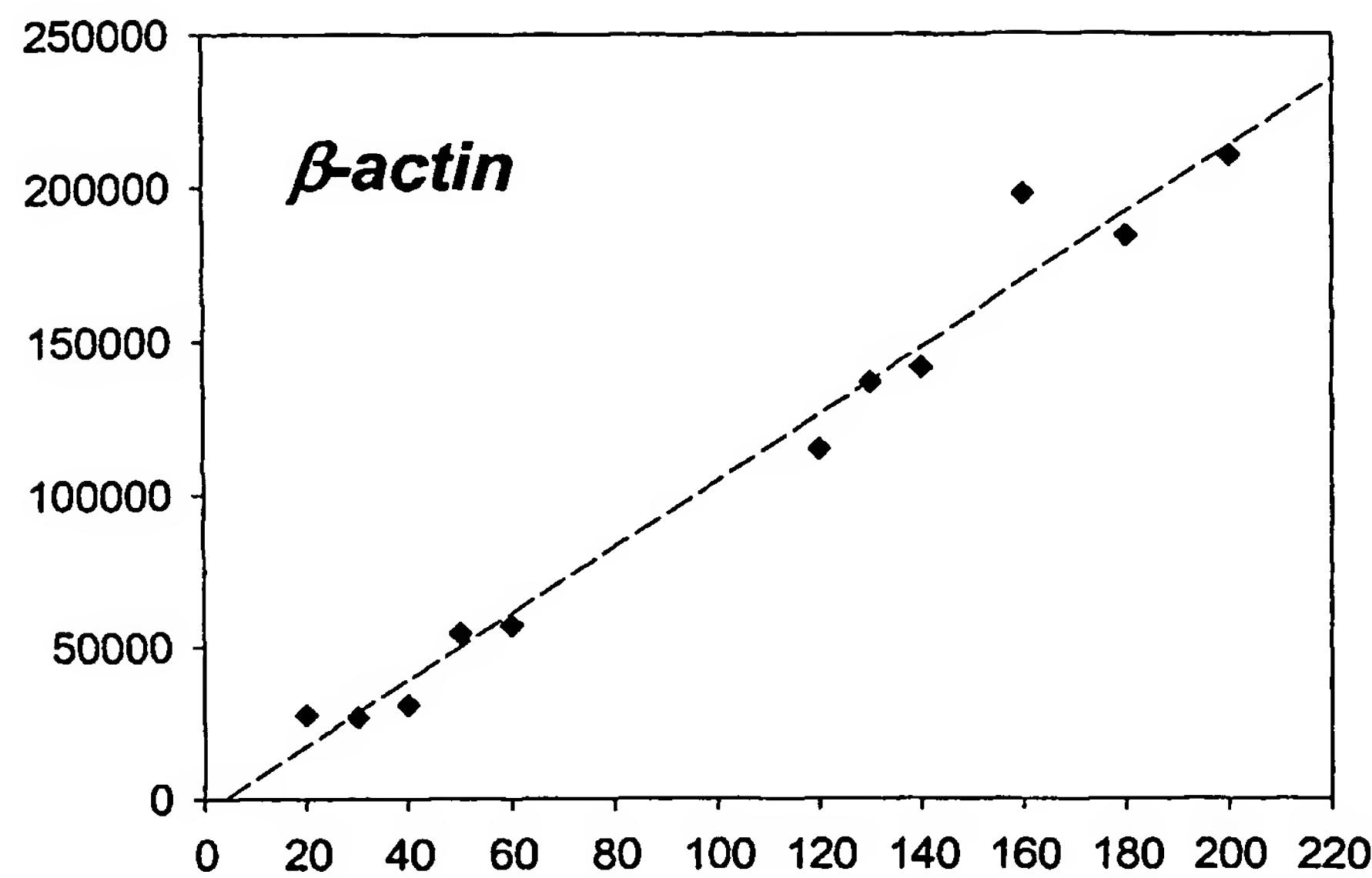


Figure 17

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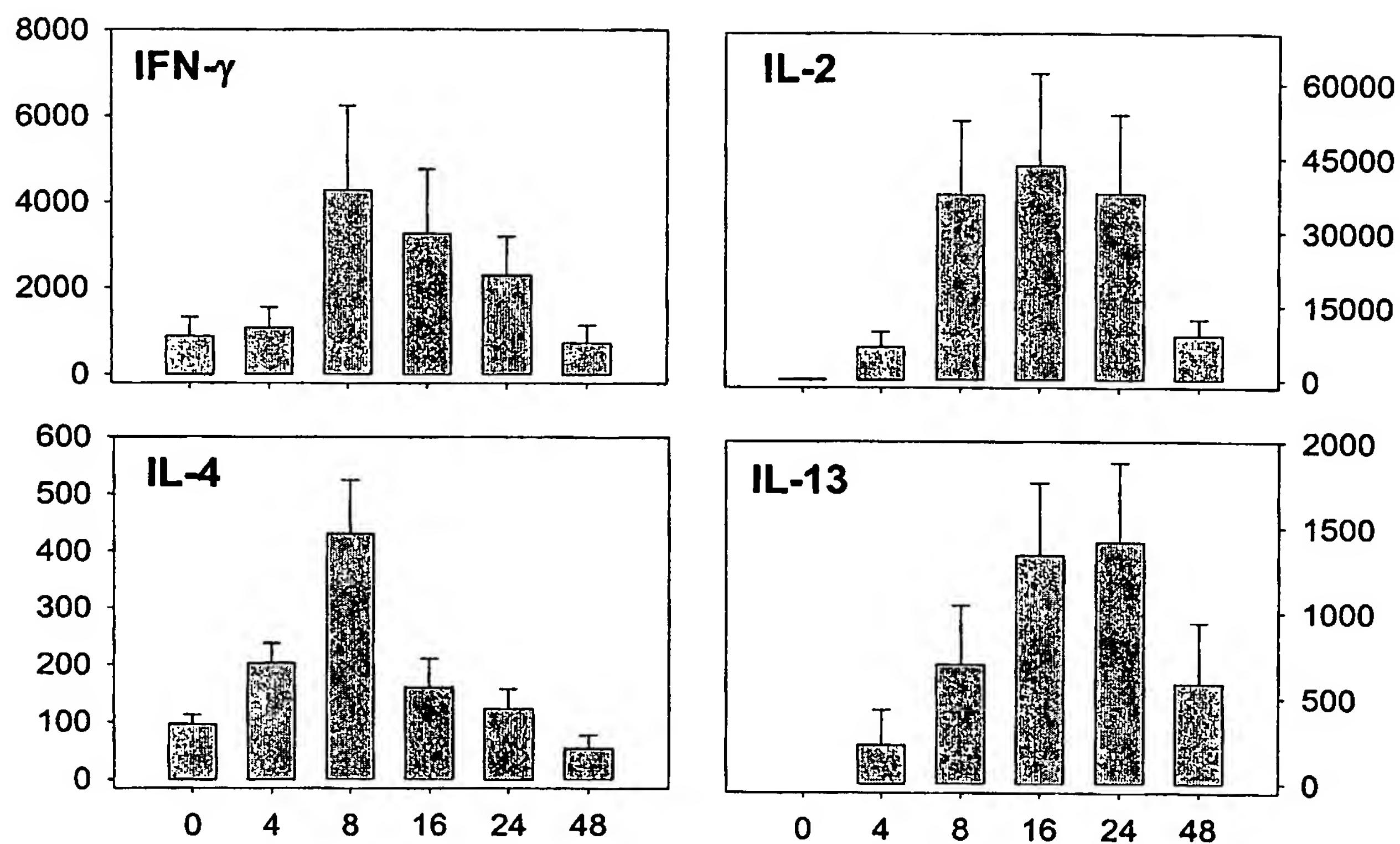


Figure 18

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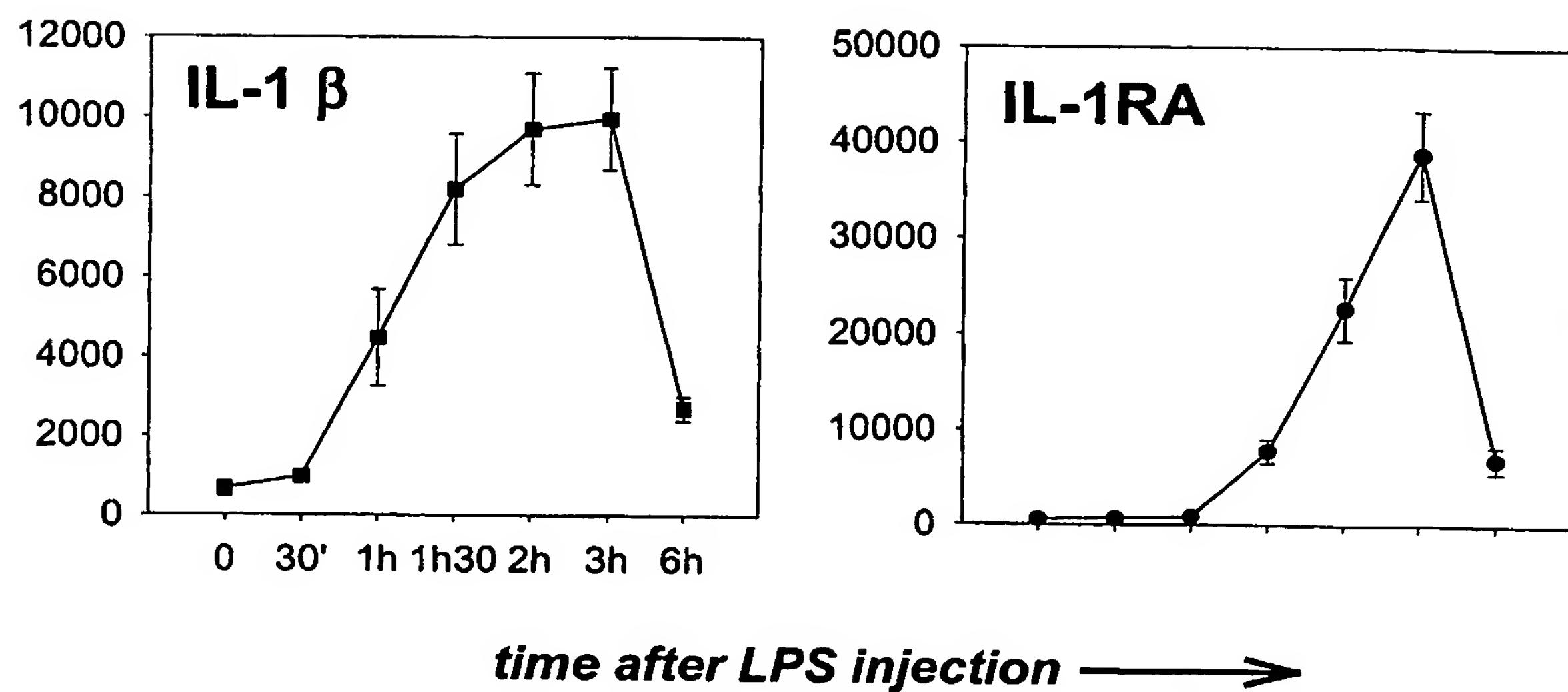


Figure 19

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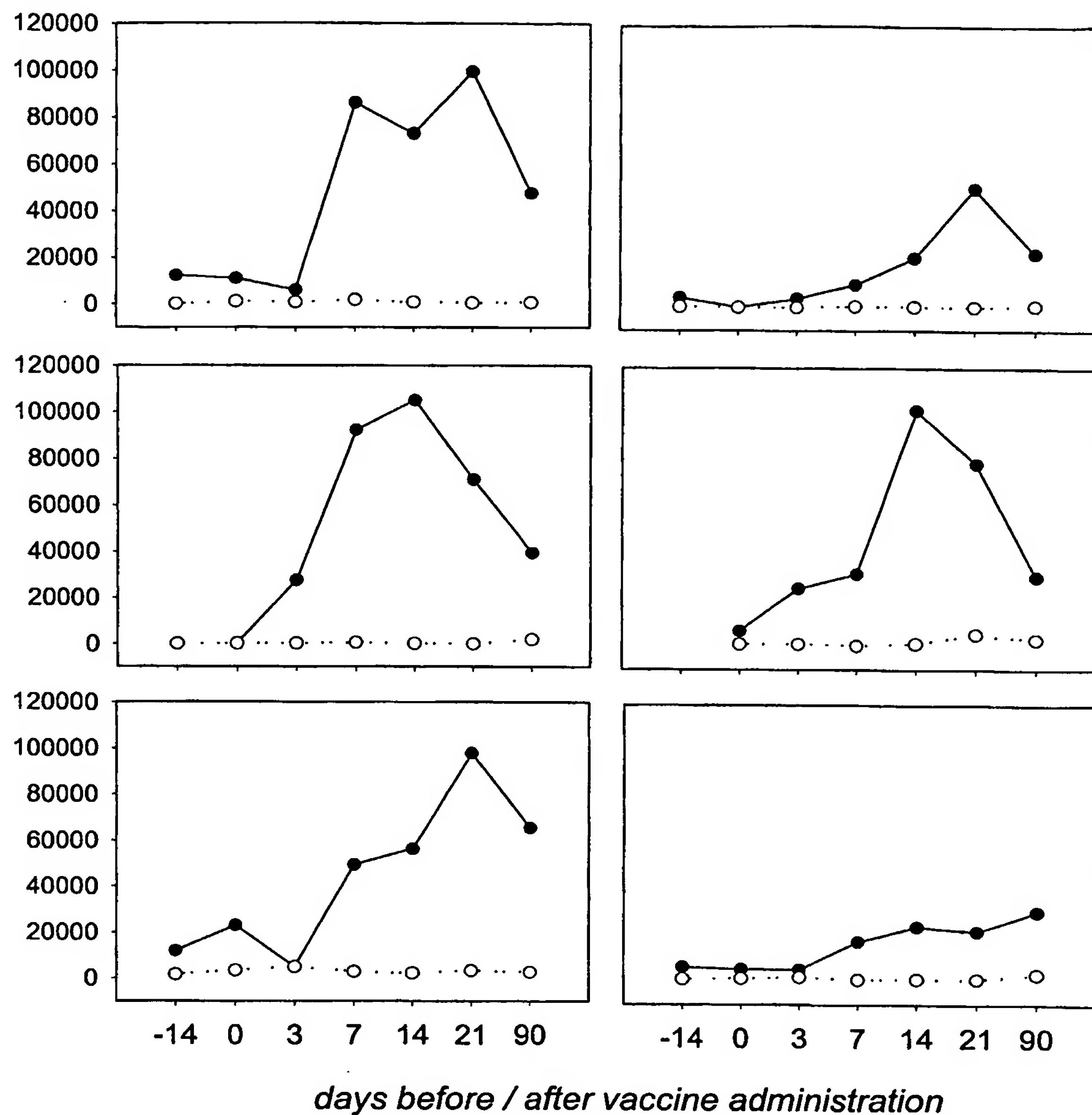


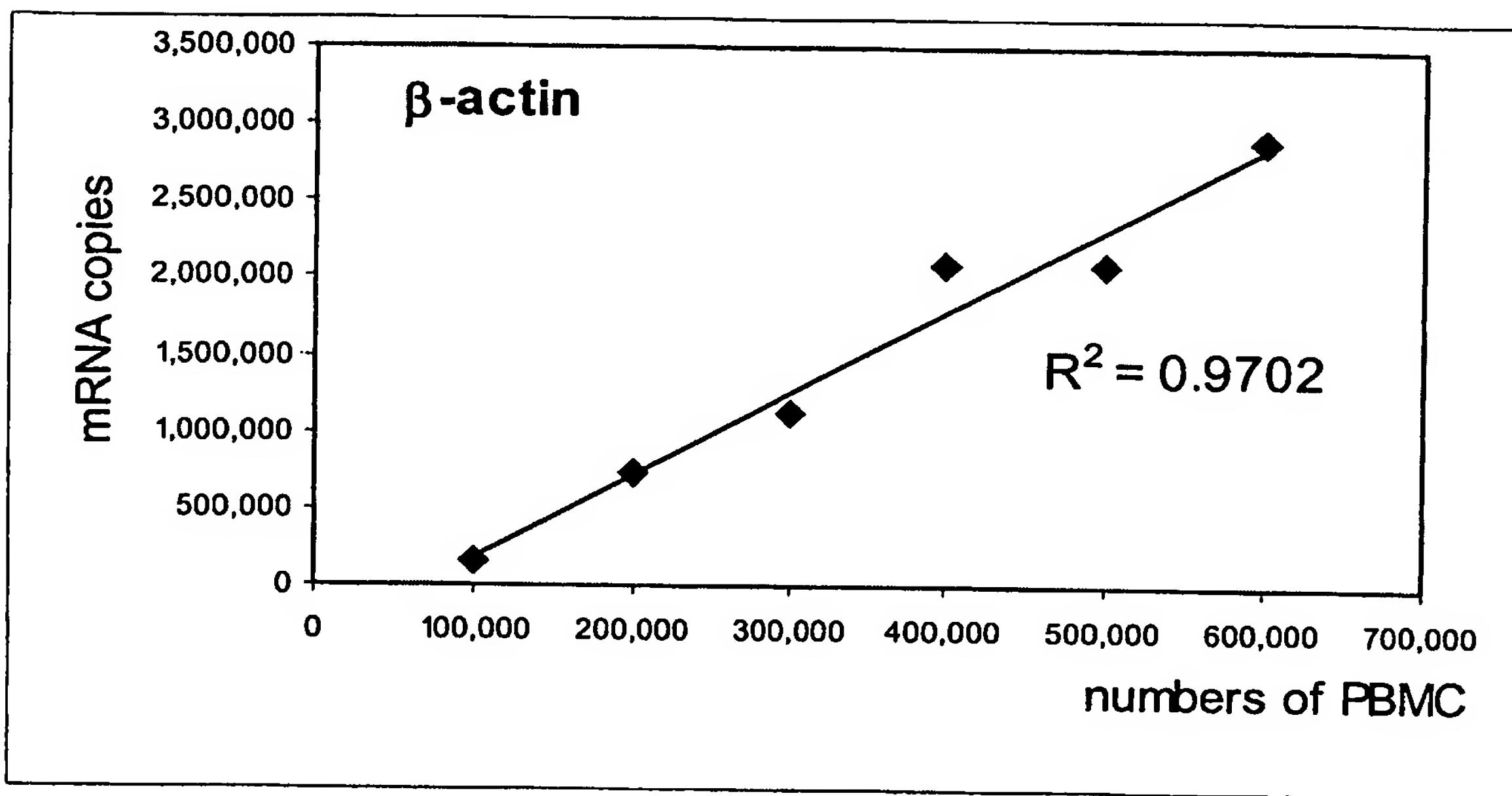
Figure 20

### Procedure to analyse blood cytokine mRNA expression

- Whole blood sample incubation in the presence the tested potential immuno-modulator (antigen, allergen, cell, xenobiotic,...)
- Blood cells lysis and mRNA stabilisation using the reagent contained in the *PAXgene* tube
- Automated mRNA extraction and preparation of the reaction mixture for one-step RT-PCR using an automated device (*Magna Pure instrument – Roche Diagnostics*)
- Quantification of cytokine mRNA levels by real-time PCR using the *Lightcycler instrument* (*Roche Diagnostics*)

Figure 21

**Automated mRNA extraction and reagent mix preparation  
on the MagNA Pure : direct correlation between starting  
cell number and found copy number**



**Figure 22**

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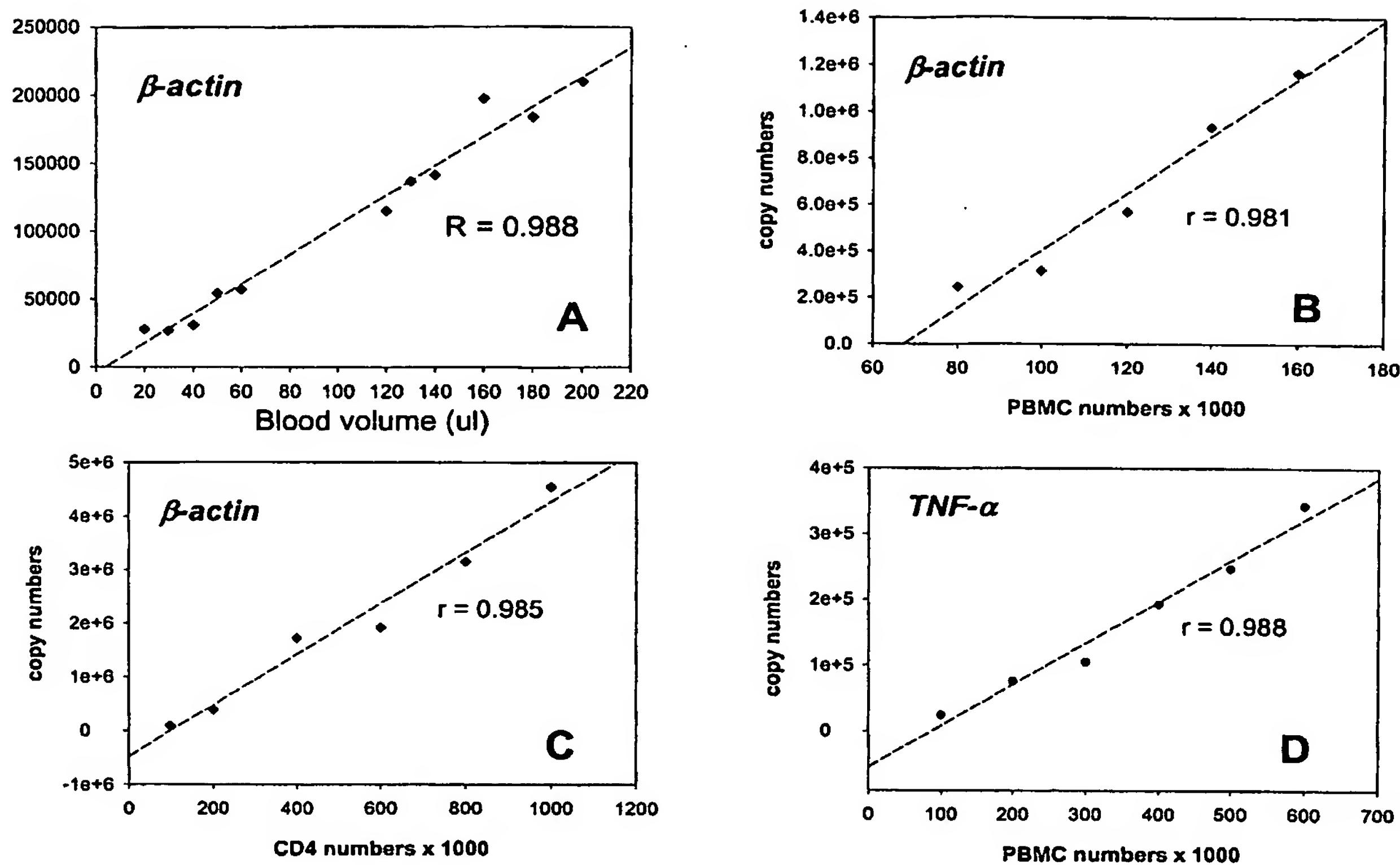


Figure 23

## Immune monitoring in cancer immunotherapy

- 7/99: melanoma of the right scapula. Excision
- 8/01: SC M+ arm, back, abdomen, right testis
- 4/02: right orchidectomy
- 4/02: pre-vaccine check-up
- 5/02: initiation of the vaccine program

Figure 24

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*In vitro stimulation of whole blood  
to assess immune response to MAGE-3*

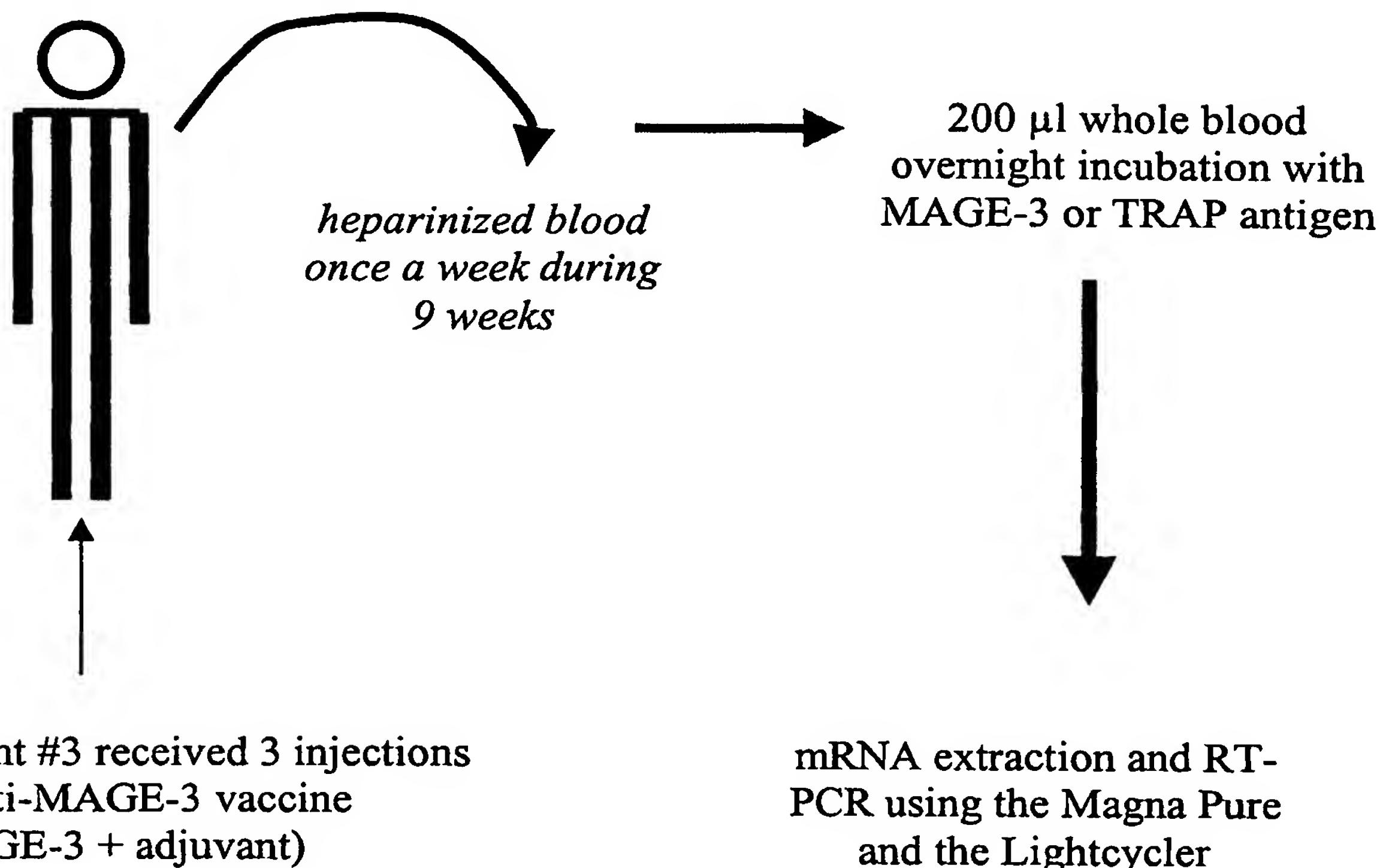


Figure 25

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**IL-2 mRNA in whole blood following MAGE-3  
vaccination in Patient #3**

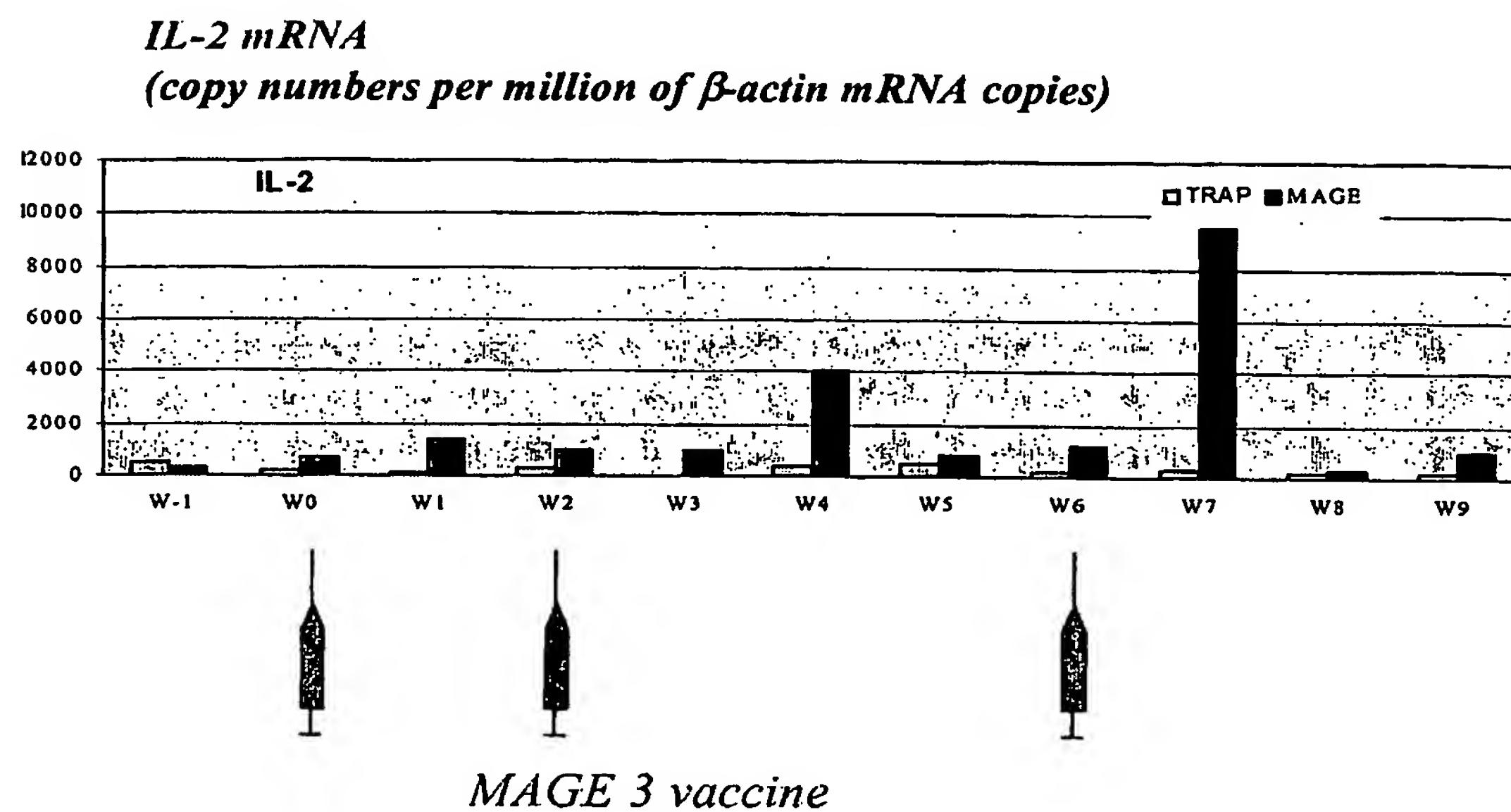


Figure 26

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*In vitro stimulation of whole blood : evaluation of immune response to allergen*

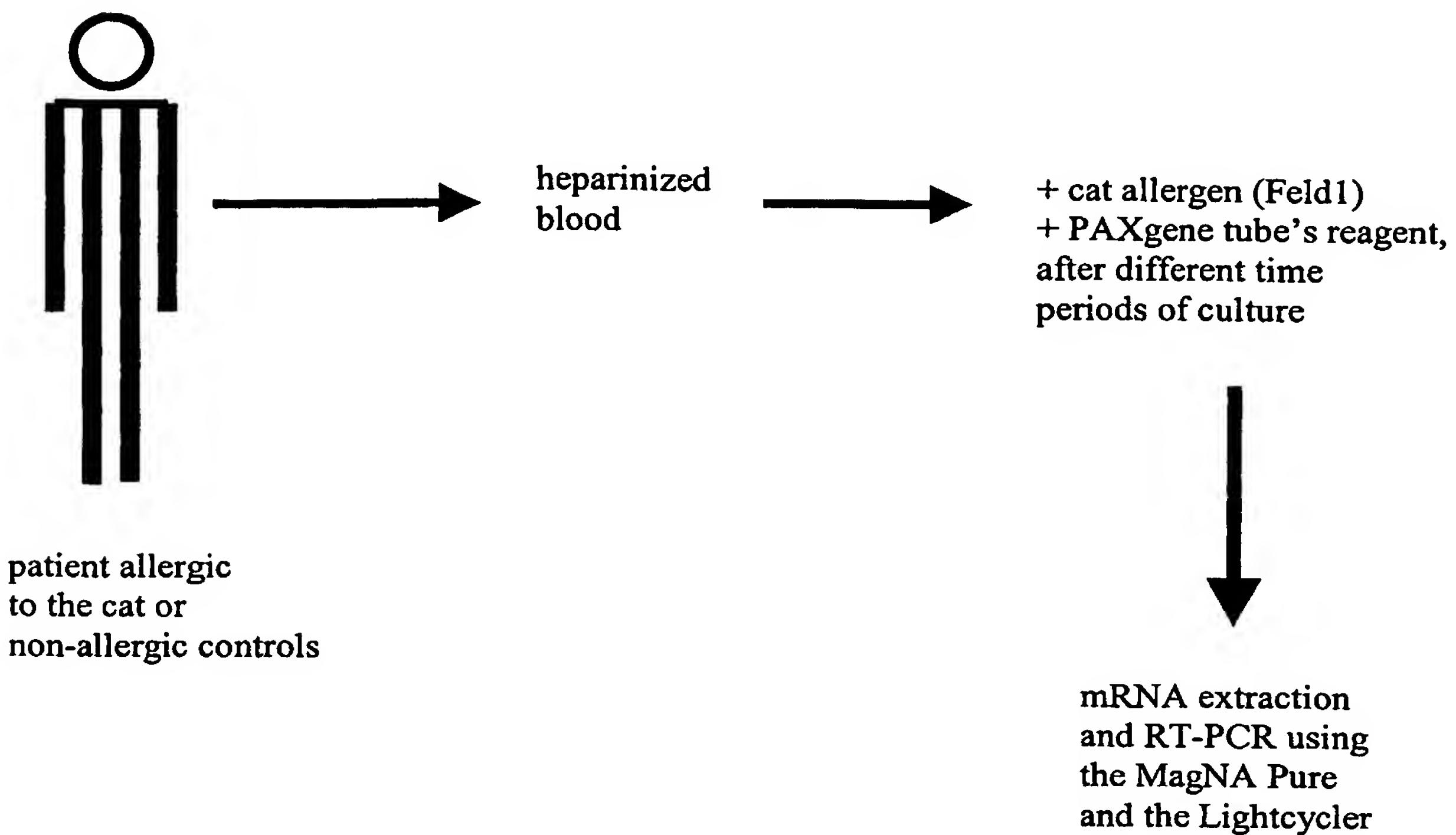


Figure 27

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***In vitro stimulation of whole blood : evaluation of immune response to Feld1***

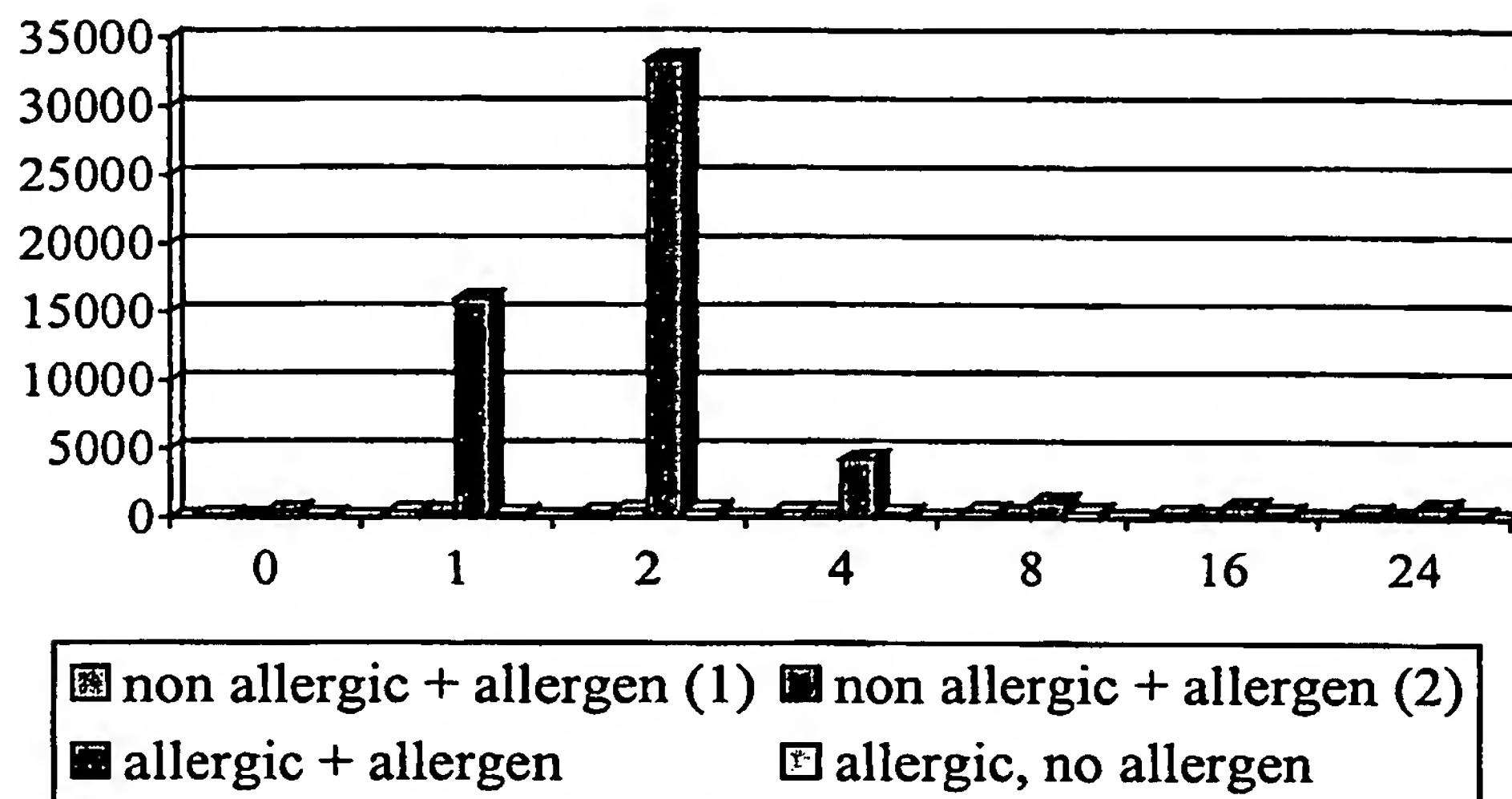


Figure 28

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*In vitro stimulation of whole blood :*  
*dose-response to Fel d1*

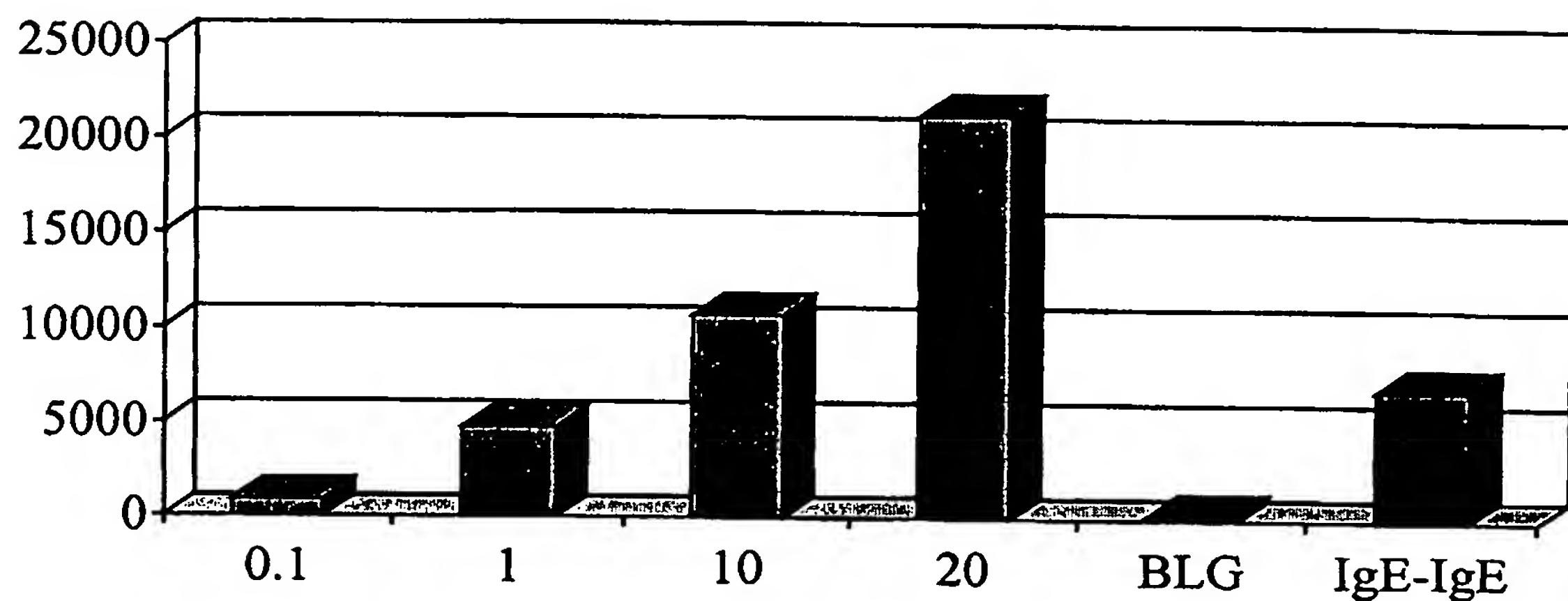


Figure 29

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**IL-4 mRNA levels after whole blood stimulation with Feld1  
are higher in allergic patients compared to healthy  
controls**

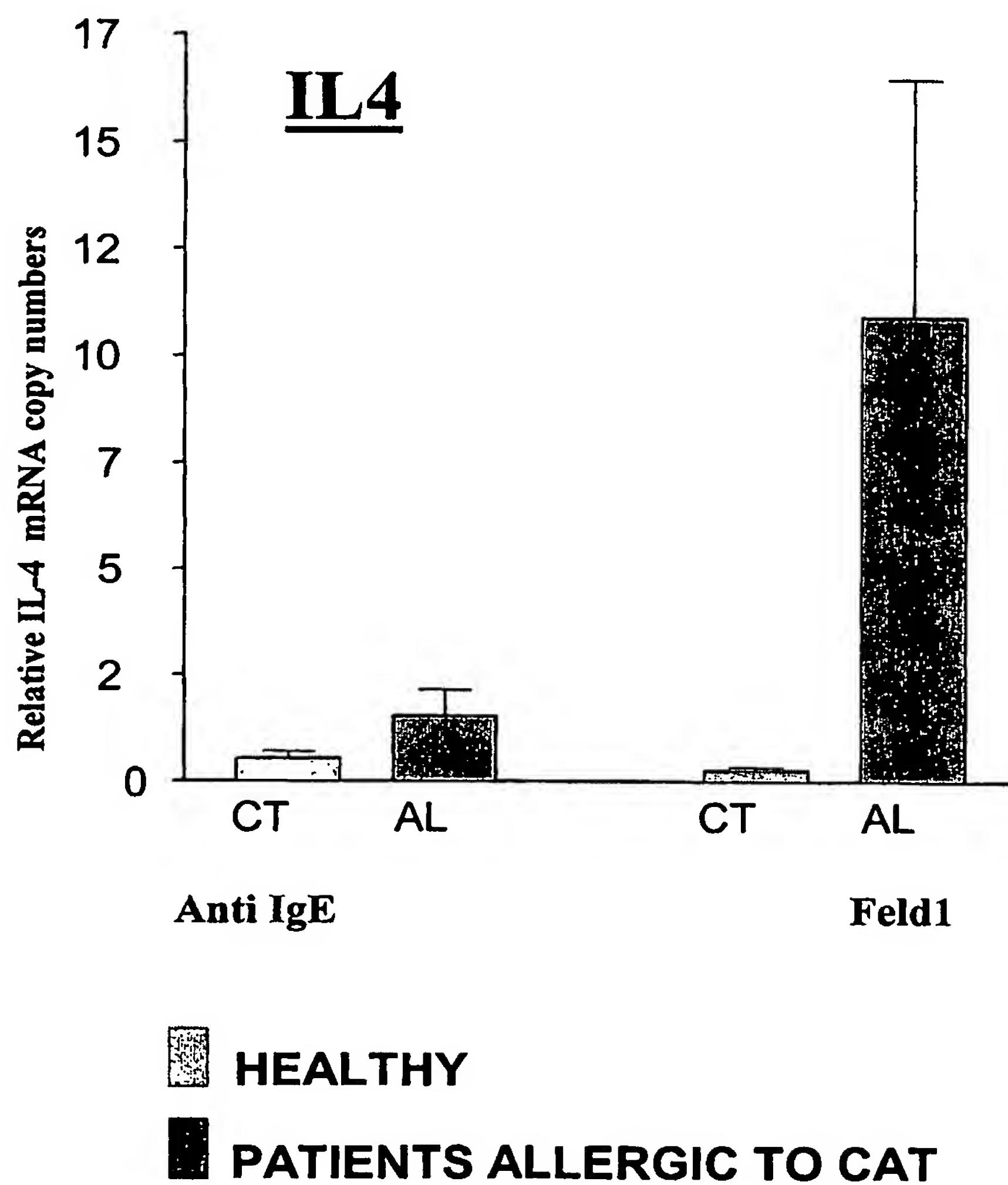


Figure 30

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*In vitro stimulation of whole blood : assessment  
of T cell response to GAD65*

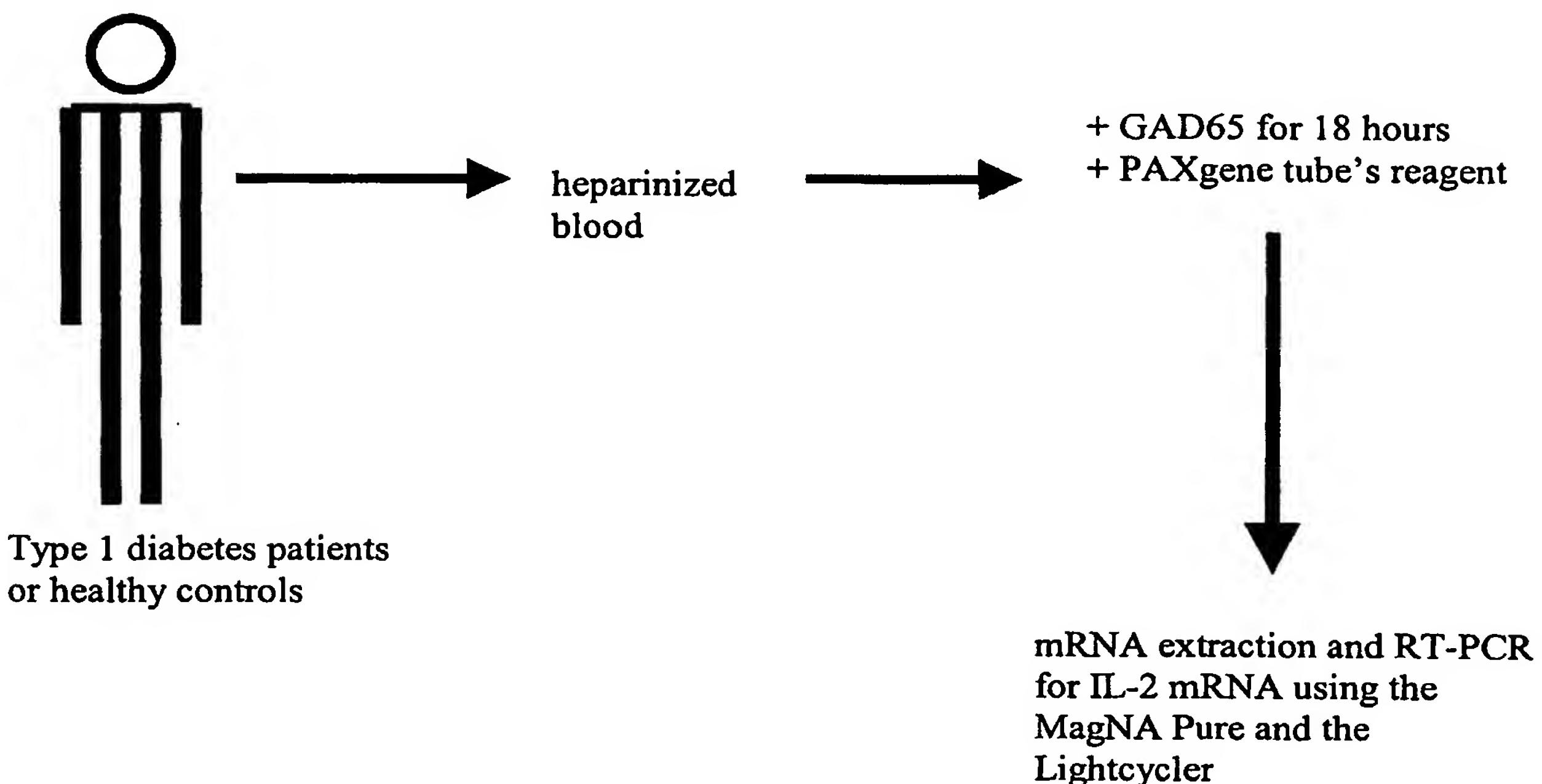


Figure 31

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*In vitro stimulation of whole blood : assessment of T cell response to GAD65*

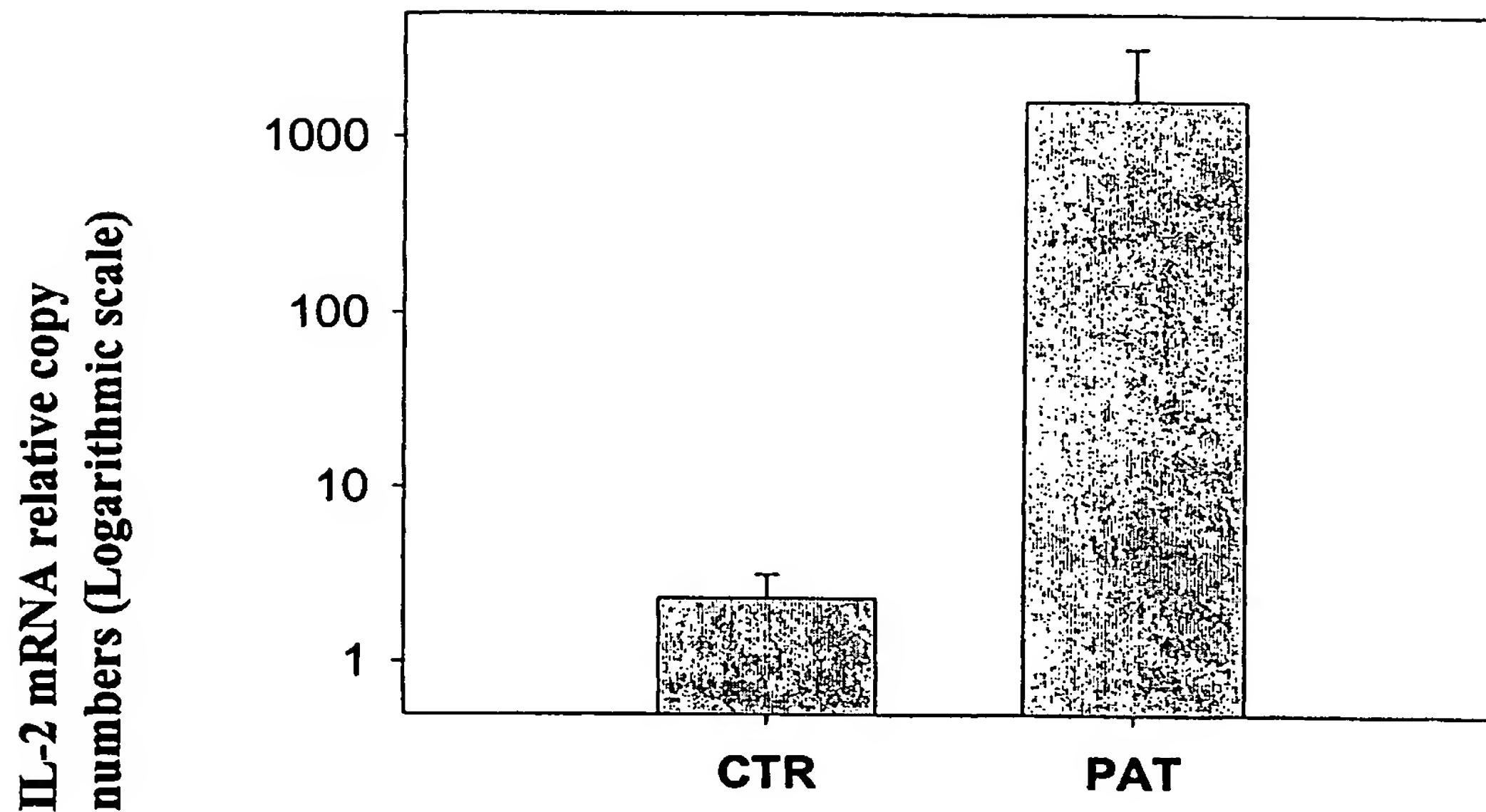


Figure 32

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**Monitoring of alloreactive immune response :  
quantification of IL-2 mRNA in a whole blood +  
dendritic cells system**

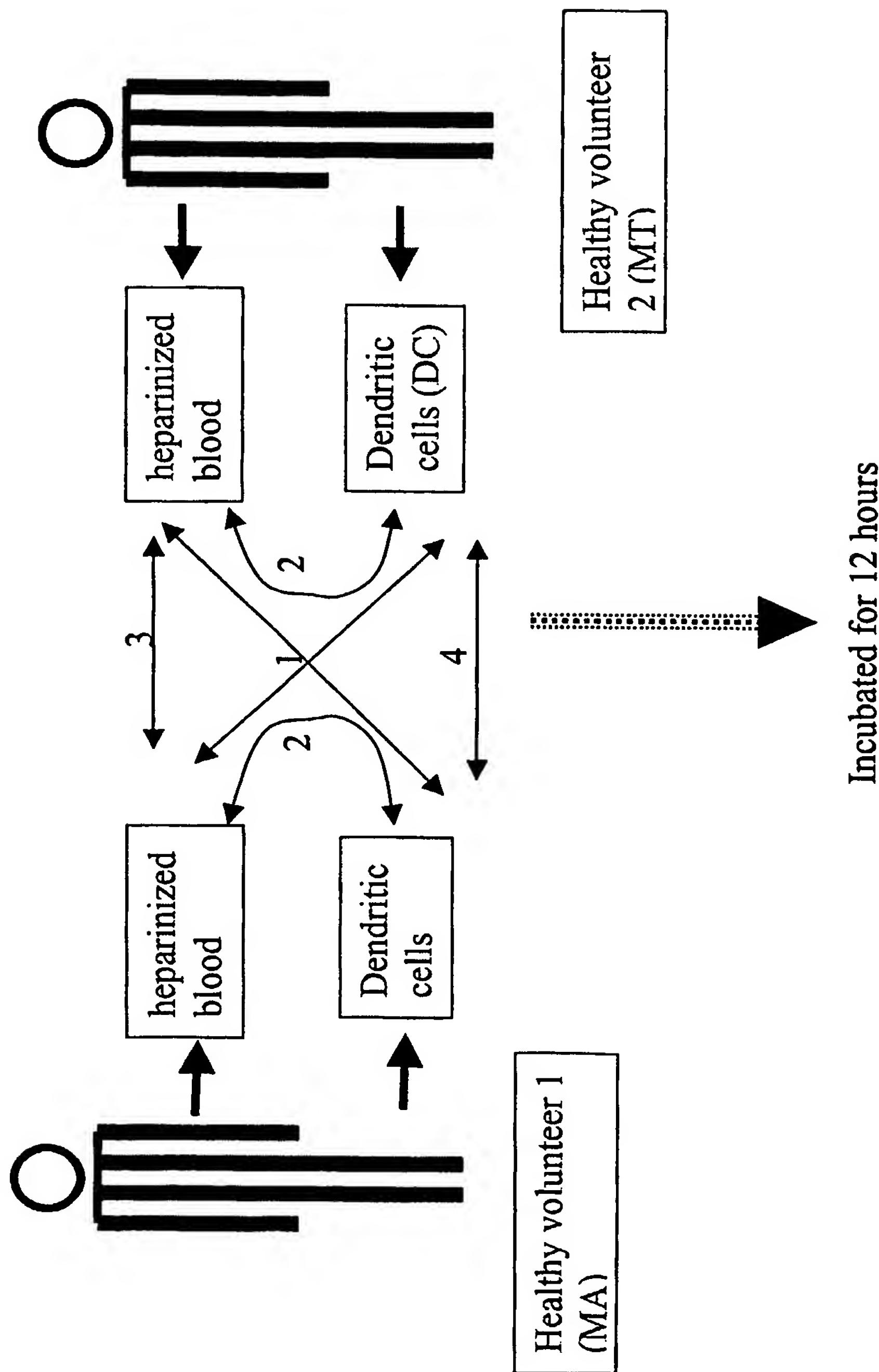


Figure 33

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**Monitoring of alloreactive immune response :  
quantification of IL-2 mRNA in a whole blood +  
dendritic cells system**

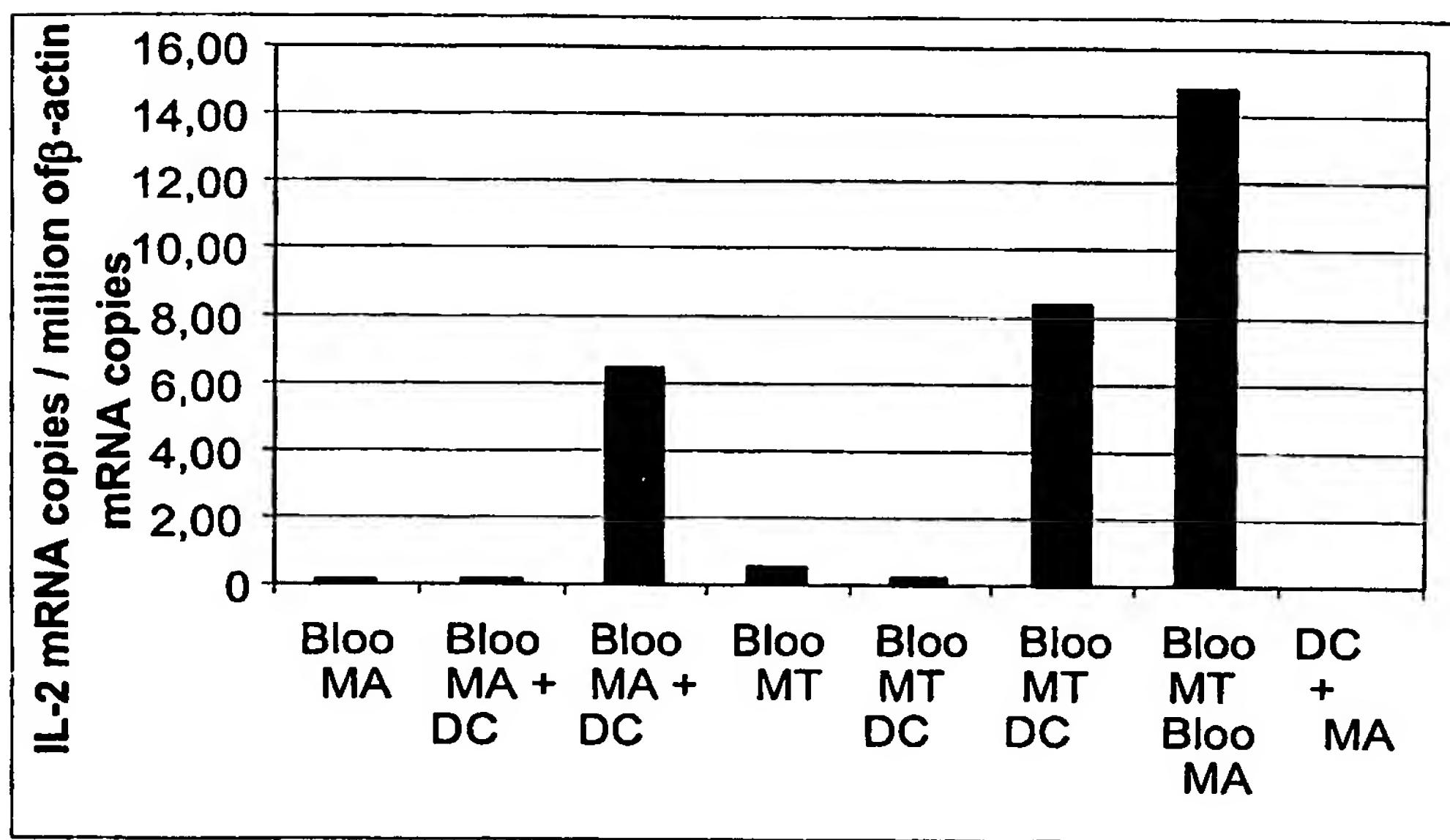


Figure 34